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Fracture resistance of human roots filled with mineral trioxide aggregate mixed with phosphate-buffered saline, with and without calcium hydroxide pre-medication

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Abstract

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Aim To compare the fracture strength of extracted human roots with apical plugs of mineral trioxide aggregate (MTA) mixed with either Ca- and Mg-free phosphate-buffered saline (PBS) or water, with and without calcium hydroxide (CH) canal pre-medication.

Methodology A total of 180 single-rooted human teeth were prepared to resemble immature roots and divided into groups ($n = 20$). The negative control received canal irrigation only, and the positive control received intracanal treatment with CH for either two or twelve weeks. MTA mixed with water was used in Group 1: (i) without CH pre-medication – MTA(W); (ii) after 2 weeks CH pre-medication – 2/52CH + MTA(W); and (iii) after 12-week CH pre-medication – 12/52 CH + MTA(W). MTA mixed with PBS was used in Group 2: (i) without CH pre-medication – MTA(PBS); (ii) after 2-week CH pre-medication – 2/52CH + MTA(PBS); and (iii) after 12-week CH pre-medication – 12/52 CH + MTA(PBS). A compressive force was applied to each root until the point of fracture. The results were analysed by the Kruskal–Wallis and Dunn's multiple comparisons tests ($P < 0.05$).

Results There was no significant difference between groups MTA(W), MTA(PBS) and 2/52CH + MTA (PBS), and all three groups were significantly ($P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively) more resistant to fracture than the negative control. Within Group 1, the samples that received two- ($P < 0.01$) and twelve-week ($P < 0.001$) CH pre-treatment were more prone to fracture than those which did not. No difference was found amongst the control groups. The roots of the MTA(PBS) group had a higher dependability ($P < 0.05$) than the MTA(W) group when compared by the Weibull modulus. The difference was also present when a 2-week CH pre-medication was used.

Conclusions Mineral trioxide aggregate mixed with Ca- and Mg-free phosphate-buffered saline had a significant strengthening effect on the fracture resistance of structurally weak roots, even when short-term calcium hydroxide pre-medication had been used. MTA mixed with water lost its strengthening effect on human roots when 2- or 12-week CH pre-treatment had been used. Use of CH dressing for up to 12 weeks had no negative effect on fracture resistance of human roots.

Keywords: calcium hydroxide, fracture resistance, mineral trioxide aggregate, phosphate-buffered saline.

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Introduction

Mineral trioxide aggregate (MTA) apexification, first described by Shabahang & Torabinejad (2000) in teeth with open apices, may not fall within the classic definition of apexification, as there is no calcified barrier or continued root development prior to filling of the apical part of the root canal with a MTA cement. More recently, other calcium silicate cements have been used for this purpose. Biological apical closure appears later, after filling of the root canal, contrary to the calcium hydroxide apexification technique, where the apical barrier is necessary to complete the root canal treatment.

It has been shown that MTA cements behave in a similar way to non-setting calcium hydroxide (CH) paste when in contact with connective tissues promoting protein denaturation and necrosis by coagulation (Yaltirik *et al.* 2004). MTA can stimulate repair because it allows cellular adhesion, growth and proliferation on its surface (Zhu *et al.* 2000), and has the ability to induce hard tissue formation when used adjacent to periradicular tissues (Shabahang *et al.* 1999).

Mineral trioxide aggregate apexification is currently the recommended technique for treating immature anterior teeth (Nicoloso *et al.* 2017, Duggal *et al.* 2017) with high pooled success rates of 94.6% being reported (Torabinejad *et al.* 2017). Two treatment protocols exist for MTA apexification. These are a two-visit treatment, with a short-term dressing of calcium hydroxide for canal disinfection, followed by the placement of an MTA apical barrier (Shabahang *et al.* 1999, Sarris *et al.* 2008), and one-visit apexification without the use of calcium hydroxide (Steinig *et al.* 2003, Simon *et al.* 2007). There appears to be no difference in success rates between the two MTA apexification protocols (Witherspoon *et al.* 2008).

Studies investigating fracture resistance of human roots filled with MTA cements reported significantly higher fracture resistance compared with roots with untreated canals (Milani *et al.* 2012, EL-Ma'aita *et al.* 2013, Bayram & Bayram 2016, Aksel *et al.* 2017, Ürkmez & Erdem 2020).

Only one study has investigated the effect of calcium hydroxide pre-treatment on fracture resistance of simulated immature human teeth filled with MTA (Karapinar-Kazandag *et al.* 2016). Those teeth were initially dressed with calcium hydroxide for 7 days and then filled with MTA. This treatment was shown to reinforce the roots to a level equivalent to mature roots that received no treatment.

Bioactivity can be described as a capacity of the material to interact with living tissues, which allows the integration of the biomaterial into the environment. Bioactivity may be measured by the ability of a material to produce carbonated apatite in the presence of a simulated body fluid (LeGeros 1991). MTA in contact with phosphate-containing fluid undergoes dissolution and releases all of its major cationic constituents, mostly calcium ions (Ca^{2+}) (Sarkar *et al.* 2005, Bozeman *et al.* 2006), which react with phosphate ions from the solution and initially form calcium phosphate, which matures into calcium-depleted carbonated apatite on the surface of MTA (Tay & Pashley 2007, Han *et al.* 2010).

The layer of carbonated apatite has been clearly shown on photomicrographs as an interfacial layer (IF) between MTA and dentine, with tag-like structures entering the dentinal tubules. This phenomenon is referred to as biomineralization (Reyes-Carmona *et al.* 2009, 2010a,b, Dreger *et al.* 2012). Han *et al.* (2010) found that calcium hydroxide and calcium carbonate were formed on the surface of MTA (mixed with water) when stored in distilled water, whereas amorphous calcium phosphate crystals were formed on the surface of the cement immersed in phosphate-buffered saline (PBS). PBS 'dressing' placed in the coronal part of the root canal over MTA mixed with water or storage of teeth filled with a MTA cement mixed with water in PBS was reported to improve biomineralization (Reyes-Carmona *et al.* 2010a,b), increase push-out bond strength of MTA (Reyes-Carmona *et al.* 2010b, de Ameida *et al.* 2014) and improve fracture resistance (Ürkmez & Erdem 2020).

It has also been demonstrated that bovine dentine in contact with an MTA cement stored in calcium (Ca)- and magnesium (Mg)-free PBS incorporated calcium and silicate (Si) ions from the MTA. Such a phenomenon may cause chemical, mechanical and structural modification of dentine, which may result in increased physical strength (Han & Okiji 2011). Similar results have been reported using human teeth (Ürkmez & Erdem 2020).

Clinically, there is a limited source of phosphate ions to react with MTA mixed with water placed in a root canal. They may be available from soft tissue fluid through diffusion from the periradicular tissues. This may be improved by mixing MTA with Ca- and Mg-free PBS. This could allow for biomineralization to take place in the parts of the root canal without direct access to the soft tissue fluid, which, in turn,

could improve the bioactivity and sealing ability of MTA (Sarkar *et al.* 2005), and additionally strengthen the roots by creation of a primary monoblock. There is also a limited evidence regarding the effect of calcium hydroxide pre-medication on the fracture resistance of teeth filled with MTA.

The primary aim of this study was to compare the fracture strength of extracted human roots with apical plugs of MTA mixed with Ca- and Mg-free PBS, and MTA mixed with water. The secondary aim was to evaluate the effect of calcium hydroxide pre-medication on fracture resistance of human roots with MTA apical plugs.

The null hypothesis to be tested is that there is no difference in the fracture resistance of human roots with MTA apical plugs regardless of the cement being mixed with Ca- and Mg-free phosphate-buffered saline or water, and whether or not calcium hydroxide pre-medication is used.

Materials and methods

One hundred and eighty extracted single-rooted human teeth were used. The collection of teeth was approved by the East of Scotland Research Ethics Service and the Tayside Medical Science Centre (Ref 2014DE04). The teeth were extracted as part of a consented treatment plan, from patients who required tooth removal for some other reason. The teeth were examined under magnification (Carl Zeiss Microscopy GmbH, Jena, Germany) and those with root caries, cracks, fractures and root resorption were rejected.

Thereafter, the crown of each suitable tooth was removed by separating it from its root with a disc (Skilldenta; Skillbond Direct Ltd., High Wycombe, UK) at the level of the labial cemento-enamel junction. The apical portion of the root was also removed in the same way, parallel to the coronal root surface, leaving a 10-mm long root. Each root canal was instrumented with size 08–15 files (NiTiFlex®; Dentsply Sirona, Ballaigues, Switzerland), and the pulp removed. The teeth were examined again under a dental operating microscope (Carl Zeiss Microscopy GmbH) at 16× magnification to exclude any cracks or fractures arising from this procedure. Teeth with cracks or fractures, sclerosed canals, more than one root canal or resorption were excluded. The remainder were stored in sterile water at room temperature for up to 60 weeks, till the start of the experiment.

Sample preparation

The root canals of the selected teeth were enlarged to create thin walls, resembling immature roots. In order to achieve this, the root canal was prepared using nickel-titanium files (sizes 15–40) with no paste lubricant, and rotary nickel-titanium files (ProTaper® Universal; Dentsply Sirona) up to a size F5, followed with Largo Peeso drills (Dentsply Sirona), sizes 1–5, with a size 5 drill (size 150) introduced 1 mm through the apical root-end (Fig. 1a). During the preparation of each root, a standardized 6 mL of 5.25% sodium hypochlorite (NaOCl) (Chloraxid®; PPH Cerkamed, Stalowa Wola, Poland) irrigant was used. Following preparation, the roots were irrigated with 3 mL of 10% citric acid (CA) and 3 mL of NaOCl and dried with paper points. The irrigating solutions were introduced into the canal system using an endodontic syringe (Monoject™; Covidien, Greenwood, SC, USA).

The roots were inspected visually, and the thinnest remaining root wall thickness was measured with a dental crown caliper, to an accuracy of 0.05 mm. The roots were then carefully divided into nine groups, of 20 roots, according to the thickness of the thinnest remaining root wall, as detailed in Table 1. The sample roots were stored in sterile water at room temperature.

Allocation to experimental groups

Prepared and grouped roots were randomly allocated to control and experimental groups as detailed in Table 2. The negative control group received irrigation only, whereas the positive control group received treatment with calcium hydroxide for either 2 weeks – 2/52 CH, or twelve weeks – 12/52 CH.

There were two experimental groups, each divided into three subgroups. In Group 1, the apical 5 mm of each root was filled with MTA mixed with water: (i) without CH pre-medication – MTA(W); (ii) after 2 weeks CH pre-medication – 2/52CH + MTA(W); and (iii) after 12-week CH pre-medication – 12/52 CH + MTA(W).

In Group 2, the apical 5 mm of each root was filled with MTA mixed with PBS: (i) without CH pre-medication – MTA(PBS); (ii) after 2-week CH pre-medication – 2/52CH + MTA(PBS); and (iii) after 12-week CH pre-medication – 12/52 CH + MTA(PBS).

Treatment procedures

To complete the preparation, the samples in the negative control group were further irrigated with 3 mL of

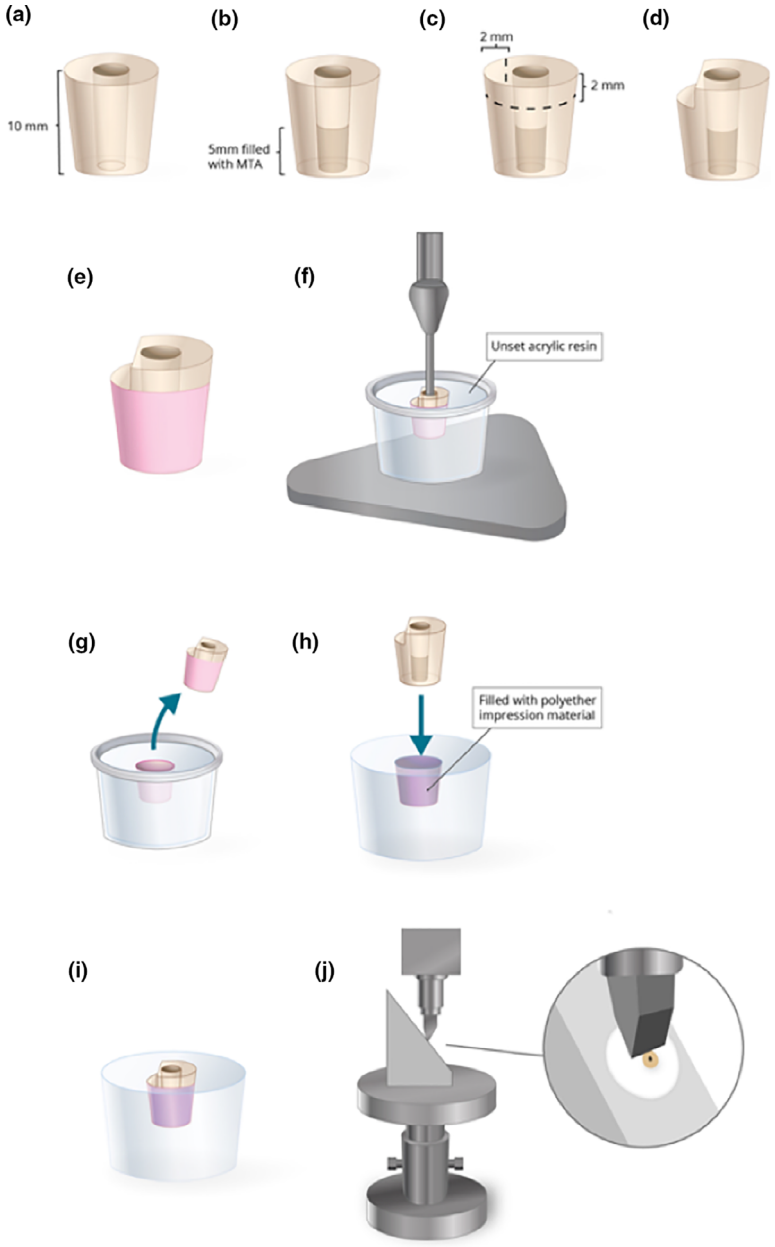

Figure 1 Preparation of samples for fracture resistance test. (a) Sample with no MTA filling, (b) sample filled with MTA apical plug, (c) orientation lines for cutting a grove and mounting the samples in acrylic moulds, (d) a groove created at the palatal/lingual aspect of each sample to facilitate placement of the tip of the chisel of the Instron® machine, (e) sample dipped in molten wax, (f) sample submerged into plastic cylindrical mould filled with freshly mixed self-curing acrylic resin to the depth indicated by the marked line, a surveyor was used to ensure perpendicular sample placement in the resin, (g) once the setting of the acrylic resin was complete, the sample was removed from the resin. Residual wax from the sample surface and the base of the created 'socket' was removed, (h) sample was re-inserted into the 'socket' filled with polyether impression material, and the excess impression material removed, (i) sample ready for fracture test after the material was allowed to fully set, and (j) sample set up in the Instron® machine using a metal jig.

Table 1 Number of roots with a specific minimal wall thickness in each group

Number of roots in each group	Minimal root thickness (mm)	Number of walls affected
1	0	1–2
2	0	1
1	≤0.2	1
3	<0.5	2
3	<0.5	1
2	0.5–0.8	1
4	0.8–1.0	1
2	1.0–1.2	1
1	1.2–1.5	1

CA and 3 mL of NaOCl and dried with paper points. The coronal part of the root was sealed with a 2-mm layer of Coltosol® F (Coltène Whaledent AG, Altstätten, Switzerland). The prepared roots were stored for 4 weeks.

During the experiment, all samples were stored for at 37°C, in 100% relative humidity in a thermostatically controlled incubator (M30C; Genlab, Widnes, UK). The relative humidity of the specimens was maintained at 100% by keeping the samples in sealed containers with 4 cotton wool rolls soaked in Ca-free and Mg-free PBS and wrapped in damp gauze.

The root canal of all samples that received CH was dried and filled with a polyethylene glycol-based CH paste (ApexCal®; Ivoclar Vivadent, Schaan, Liechtenstein) using a syringe applicator. The coronal portion of the root canal was sealed with a 2-mm-thick temporary cement (Coltosol® F). Depending on the group allocation, the samples were stored for either 2 or 12 weeks. After that time, the temporary cement was removed, and the teeth irrigated with 3 mL of CA and 3 mL of NaOCl and dried with paper points. Prior to MTA placement in groups without CH pre-medication, the samples were irrigated with 3 mL of CA and 3 mL of NaOCl, to ensure the same total quantity of irrigation solutions were used for all samples, and dried with paper points.

In the experimental groups, white MTA (white ProRoot™ MTA; Dentsply, Tulsa Dental, OK, USA) was mixed with sterile water (Group 1) or Ca-free and Mg-free PBS (Group 2), using 0.33 g of liquid and 1 g of MTA. The PBS consisted of 136.4 mM NaCl, 2.7 mM KCl, 8.2 mM NaH_2PO_4 and 1.25 mM KH_2PO_4 in deionized water (pH 7.2) was used. MTA was used to fill the apical 5 mm of the roots (Fig. 1b). Increments of cement were transferred into the root canal using a disposable MTA carrier and condensed manually using a plugger (B&L Condenser; B&L Biotech Inc., Fairfax, VA, USA). During MTA condensation, ultrasonic energy (Satelec P5 Newtron; Acteon, Saint Neots, UK and B&L Ultrasonic endodontic tip; B&L Biotech Inc.) was applied to the plugger for 5 s. A moist cotton pellet was placed over the MTA (cotton pellets were soaked in either sterile water or Ca- and Mg-free PBS, to match the liquid used to mix the MTA) and sealed with temporary cement (Coltosol® F). The samples were stored for 4 weeks.

Preparation of samples for fracture test

The temporary restoration was removed from the coronal portion of the roots of each specimen. A 2-

Table 2 The control and experimental groups and summary of treatment they received

Group name	Subgroups (n = 20)	Treatment received
Negative control	Irrigation only	Irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl
Positive control	2/52 CH	CH dressing for 2 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl
	12/52 CH	CH dressing for 12 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl
Group 1	MTA(W)	Irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm water-mixed MTA apical plug
	2/52 CH + MTA(W)	CH dressing for 2 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm water-mixed MTA apical plug
	12/52 CH + MTA(W)	CH dressing for 12 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm water-mixed MTA apical plug
Group 2	MTA(PBS)	Irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm PBS-mixed MTA apical plug
	2/52 CH + MTA(PBS)	CH dressing for 2 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm PBS-mixed MTA apical plug
	12/52 CH + MTA(PBS)	CH dressing for 12 weeks, followed by irrigation with 3 mL of 10% CA and 3 mL of 5.25% NaOCl, and placement of a 5 mm PBS-mixed MTA apical plug

mm deep grove was cut on the palatal/lingual aspect of the coronal root-end using a diamond fissure bur in a water-cooled high-speed handpiece (Fig. 1c,d).

Prepared roots from each subgroup were randomly selected to undergo fracture resistance tests. The roots were dipped in molten wax (Modelling Wax; Associated Dental Products Ltd., Swindon, UK) for 1s up to the line marked 2 mm from the coronal root surface (Fig. 1e). This resulted in deposition of a 0.2–0.3 mm layer of wax. Thereafter, the roots were submerged into plastic cylindrical moulds filled with freshly mixed self-curing acrylic resin (Orthoresin; Dentsply DeTrey, Hanau-Wolfgang, Germany) until there was a 2-mm gap between the coronal portion of the sample and the top of the resin. A surveyor was used to ensure perpendicular sample placement in the resin (Fig. 1f).

Once the setting of the acrylic resin was complete, the samples were removed from the resin (Fig. 1g). Residual wax at the base of the created 'socket' was removed from the acrylic mount using boiling water followed by drying with a 3 in 1 syringe. Wax from the root ends was removed with a sharp spoon excavator, and any further residue was removed by rubbing the specimen end with gauze after dipping the root in boiling water for 1 s to soften the wax. The acrylic cylinders were removed from the plastic moulds and their 'sockets' were filled with polyether impression material (Impregum™; 3M ESPE, Seefeld, Germany). Prior to the impression material setting, the roots were re-inserted into the 'socket', and the excess impression material removed (Fig. 1h). The material was then allowed to set fully (Fig. 1i). The fracture resistance tests were undertaken the following day prior to which the completed specimens were stored dry at room temperature. A variant of this procedure applied to the root canals of teeth with no MTA apical plug where paper points were placed in the root canals prior to the roots being dipped in wax, placed in the orthodontic resin and re-inserted into the 'socket' filled with polyether impression material. This ensured that no material entered the root canal.

Fracture resistance test

To carry out the fracture resistance test, a metal jig was designed and fabricated for the purpose of this study. This permitted the prepared tooth specimens to be loaded by the tip of a chisel to destruction at 130° to the long axis of the root in a lingual-labial direction, using an Instron® Universal (4449; Instron, High Wycombe, UK) testing machine at a crosshead speed of 5 mm/min. This experimental setup is shown in detail in Figs 1(j) and 2.

All specimens were tested to fracture, and the maximum force at fracture (*F*-max) was recorded in Newton (N). The roots were then removed from the acrylic 'sockets' and inspected for the type of fracture: split or comminuted, and three depths of fracture: at the level of the cylinder, into the cylinder and vertical root fracture.

Element mapping

Two roots per group, which had not undergone the fracture resistance testing, were used for the analysis. Each root was sliced perpendicular to its long axis. The cut surface was polished for 20 s using a fine sharpening stone, etched for 20 s with 37% phosphoric acid and mounted on aluminium stubs for

Figure 2 Sample mounting in the Instron® Testing Machine. Specimens fixed in a jig, with a chisel-shaped tip applied at 130° to the long axis of the tooth in a lingual-labial direction.

analysis. Four randomly selected samples per group underwent element mapping using a scanning electron microscopy (SEM) (JEOL JSM-5600; JEOL USA Inc., Peabody, MA, USA) with a EDX detector (INCAx-sight; Oxford Instruments, Abingdon, UK). The dentine–MTA interface and/or dentine (for groups with no MTA plug) were analysed at an accelerating voltage of 20 kV. Mapping of Ca, Si and phosphorus (P) elements in the dentine–MTA interface and/or dentine was recorded. The mapping images for all samples were superimposed on the original SEM image, and the MTA margin was marked using an Adobe® Photoshop (Adobe Systems Inc., San Jose, CA, USA) tool. Scanning electron micrographs of dentine and dentine–MTA interface of the samples that underwent element mapping were taken at various magnifications (100–1000×).

Statistical analysis

The mean *F*-max and standard deviation were calculated for each subgroup for the fracture resistance tests. D'Augusto and Pearson omnibus normality test revealed that the data were normally distributed in all groups. These findings were analysed using Kruskal–Wallis test with post hoc testing, by the Dunn's multiple comparisons test and the Weibull cumulative distribution function (Weibull 1951). A chi-square test was undertaken to compare the types and depths of root fracture seen across the groups. The mean F-max and standard deviation were calculated for each type and depth of fracture. These findings were analysed using ANOVA test. All statistical testing was performed to a level of statistical significance of $P < 0.05$. Based upon the experimental data, a Power Calculation made using GraphPad StatMate (version 2.00; GraphPad Software Inc., San Diego, CA, USA) showed 95% statistical power to detect a difference between mean fracture strengths of 61 MPa and 90% power to detect a difference of 55 MPa.

Results

Table 3 and Fig. 3 show the mean maximum fracture strengths calculated for each group and their standard deviations.

The results of the Kruskal–Wallis test for the mean F-max between all groups showed highly significant differences amongst the groups ($P < 0.0001$). The Dunn's multiple comparisons test highlighted several significant differences ($P < 0.05$) amongst groups, which are also presented in Table 3.

There was no significant difference between subgroups MTA(W), MTA(PBS), 2/52CH + MTA(PBS) and 12/52CH + MTA(PBS), and all of them but subgroup 12/52CH + MTA(PBS) were significantly more resistant to fracture than the negative control ($P < 0.01$, $P < 0.05$, $P < 0.05$, respectively). Within Group 1, the samples that received two- ($P < 0.01$) and twelve-week ($P < 0.001$) CH pre-treatment were significantly more prone to fracture than those which did not. The samples from group 12/52 CH were significantly more resistant to fracture than samples in group 12/52CH + MTA(W) ($P < 0.05$). Similar, samples in group 2/52CH + MTA(PBS) were significantly more resistant to fracture than samples in group 2/52CH + MTA(W) ($P < 0.05$). No significant difference was found amongst the control groups.

Table 3 Summary of the mean maximum fracture strengths (*F*-max) in Newton, their standard deviation, and the statistically significant differences between the subgroups highlighted by the Dunn's multiple comparisons test

Subgroup No	Subgroup	*F*-max (N)	Standard deviation	Statistically significantly different than subgroup
1	Irrigation only	462.34	205.42	4**, 7*, 8*
2	2/52 CH	598.90	194.67	
3	12/52 CH	706.56	240.66	6*
4	MTA(W)	920.41	403.05	1**, 5**, 6***
5	2/52 CH + MTA(W)	446.68	201.07	4**, 7*, 8*
6	12/52 CH + MTA(W)	409.17	211.52	3*, 4***, 7**, 8**
7	MTA(PBS)	852.61	375.70	1*, 5*, 6**
8	2/52 CH + MTA(PBS)	832.36	328.73	1*, 5*, 6**
9	12/52 CH + MTA(PBS)	513.87	275.44	

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

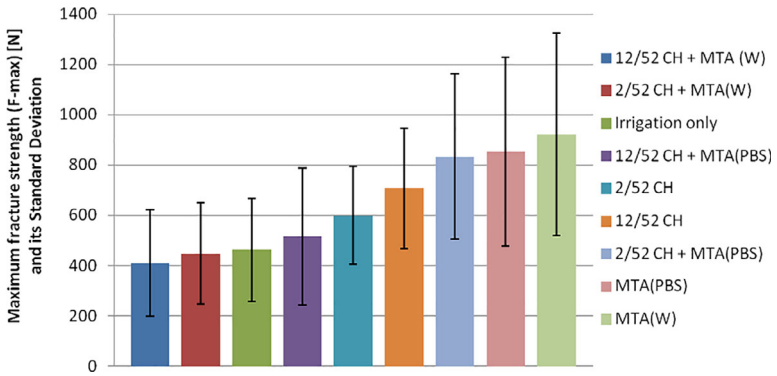

Figure 3 Summary of the mean maximum fracture strengths (*F*-max) in Newton, calculated for each group and their standard deviation.

The outcome of a Weibull analysis of the fracture strength data is given in Table 4. The analysis revealed statistically significant differences ($P < 0.05$) in dependability (the quality of being reliable) of the specimen to fracture amongst several groups, which are presented in Table 4. It demonstrated that group MTA(W) had poorer dependability ($P < 0.05$) to fracture than groups 2/52 CH + MTA(PBS) and MTA (PBS) (Fig. 4) at higher failure probability of values.

The most common fracture mode was the split root fracture (69% overall) in all groups apart from 12/52 CH + MTA(PBS). The chi-square calculation two modes of fracture revealed that the experimental groups and the fracture modes (Chi-square value of 19.06, $P = 0.015$) and the fracture depths (Chi-square value of 50.46, $P = 0.00002$) were not independent. The majority of roots suffered deep fractures (72.5% overall): 45% of roots fractured below the cylinder level, and 27% had vertical root fracture. Only 27.5% of roots fractured above the cylinder.

The result of the one-way ANOVA test revealed no significant difference between the mean F-max for fracture types ($P = 0.79$) and fracture depths ($P = 0.0538$).

It was possible to distinguish the interfacial layer between the cement and dentine in the SEM examination of samples filled with MTA mixed with PBS (Group 2), and a 50–200 μm layer of altered dentine adjacent to the cement (Fig. 5a–c). No such a layer could be observed in Group 1. Diffusion of Si, P and Ca elements from the MTA root fillings into dentine was found in groups MTA(W) (Fig. 6a–c), 2/52

Table 4 Summary of the Weibull moduli and the significant differences between the subgroups

Subgroup No	Subgroup	Weibull distribution	Number of specimens	Weibull modulus (standard error)	Characteristic strength the force at which 63.2% of specimens will fail	Statistically significantly different than subgroup
1	Irrigation only	0.81	18	2.38 (0.28)	528.30	
2	2/52 CH	0.94	17	2.97 (0.19)	674.15	4*, 8*
3	12/52 CH	0.95	18	2.37 (0.14)	813.52	5*, 6*, 9*
4	MTA(W)	0.97	18	1.87 (0.01)	1070.00	2*, 5*, 7*, 8*
5	2/52 CH + MTA (W)	0.96	18	2.20 (0.12)	509.57	3*, 9*
6	12/52 CH + MTA (W)	0.96	18	2.04 (0.11)	465.23	3*
7	MTA(PBS)	0.93	17	2.22 (0.16)	974.25	4*
8	2/52 CH + MTA (PBS)	0.98	18	2.31 (0.10)	951.82	2*, 4*
9	12/52 CH + MTA (PBS)	0.96	18	1.93 (0.10)	586.80	3*

*$P < 0.05$.

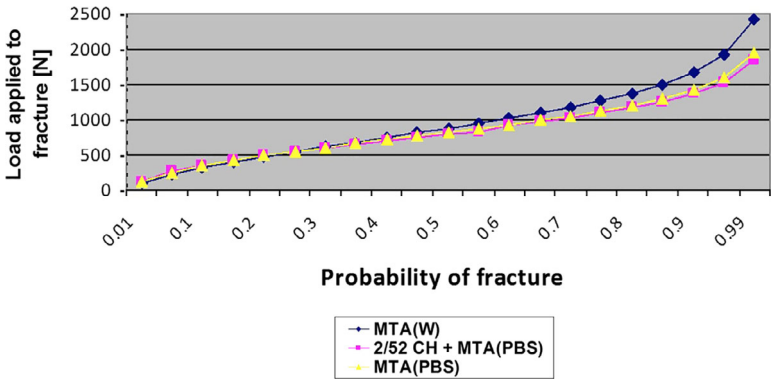

Figure 4 The chart illustrates significant differences between the probability of failure in relation to the applied load in Newton between groups: MTA(W) versus 2/52 CH + MTA(PBS) and MTA(PBS).

CH + MTA(PBS) (Fig. 6d–f) and 12/52 CH + MTA (PBS) (Fig. 6g–i). Diffusion of Si only could be observed in MTA(PBS) (Fig. 6j). No element diffusion could be detected in all other groups.

Discussion

When interpreting the results of fracture resistance studies, it should be understood that the experimental setup simplifies the highly specialized periodontal support tissues and the physiological forces applied to teeth. The forces used in laboratory studies are compressive and applied progressively. Clinically, tooth fracture is more likely to be caused by a sudden impact or by catastrophic failure after prolonged fatigue forces cause crack propagation of the tooth structure. The forces may also be applied at varying angles to amounts of residual coronal tooth tissue offering fracture resistance.

A sample size of 20 was chosen based on previous similar studies which used 12–15 samples per group (Milani *et al.* 2012, EL-Ma'aita *et al.* 2013, Bayram & Bayram 2016, Evren *et al.* 2016, Çiçek *et al.* 2017, Ürkmez & Erdem 2020).

The roots in the present study were mounted for the fracture test to simulate as closely as possible the anatomical position in the mouth. It has been previously shown (Isidor *et al.* 1996, Sirimai *et al.* 1999, Rees 2001, Soares *et al.* 2005) that simulation of the PDL and the supporting tissues is crucial when testing fracture resistance to correctly determine the stress distribution and fracture modes. In the present study, acrylic resin was used to simulate bone, as such a material has been shown to be able to reproduce the capacity of bone to withstand forces of mastication, the PDL was simulated using a polyether impression material as recommended by Soares *et al.* (2005). The bone and PDL simulation procedure required the roots to be dry, although no attempt was made to desiccate them. The fracture resistance test was also conducted in dry conditions, which may have an impact on the fracture resistance of dentine (Kahler *et al.* 2003). A variation of this technique was used in other studies (Zarei *et al.* 2013, EL-Ma'aita *et al.* 2013, Evren *et al.* 2016, Çiçek *et al.* 2017, Aksel *et al.* 2017, Ürkmez & Erdem 2020).

A 2-mm gap between the cemento-enamel junction and the top of the resin simulated the anatomical spacing found between the bone and the CEJ, to allow a focus on loading the roots rather than the tooth, as suggested by Wilkinson *et al.* (2007), and used in other studies (Milani *et al.* 2012, EL-Ma'aita *et al.* 2013, Evren *et al.* 2016, Bayram & Bayram 2016, Çiçek *et al.* 2017, Aksel *et al.* 2017, Ürkmez & Erdem 2020).

A 2-mm deep groove was cut on the palatal/lingual aspect of the coronal root-end in order to both prevent the chisel slipping and also to standardize crack propagation according to the Griffith Criterion for Failure (Griffith 1921). The specimens were loaded with the tip of a chisel at 130° to the long axis of the tooth in a lingual-labial direction, simulating the inter-incisal angle in a Class I incisal relationship in humans. Such an approach has also been adopted previously (Hemalatha *et al.* 2009, Evren *et al.* 2016, Çiçek *et al.* 2017).

It was not feasible to collect only immature teeth; therefore, the single-rooted teeth obtained were of different type, ages and stages of root development. This should be considered when interpreting the results of this study (Kishen 2015). They were, however, standardized according to the length of the root and the size of the root canal. Additionally, careful allocation

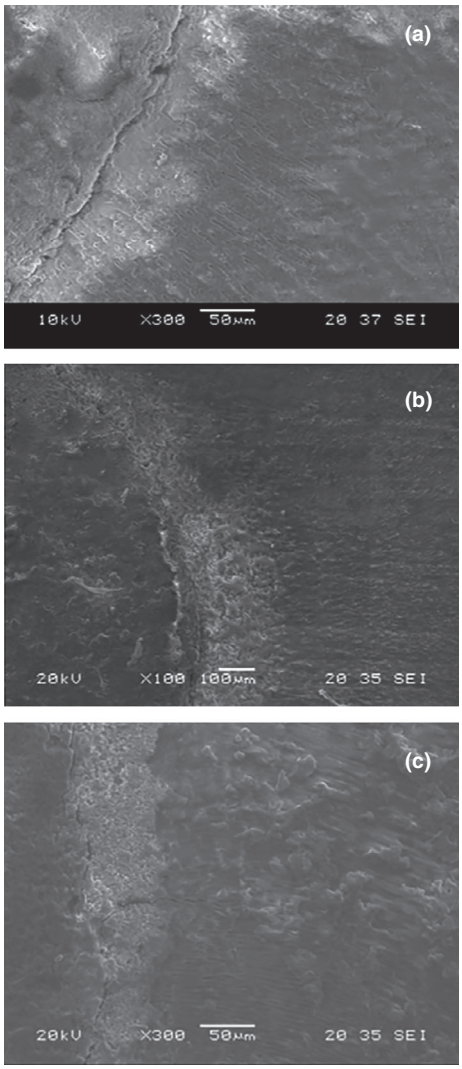

Figure 5 The scanning electron microscopy image of: (a) group MTA(PBS), (b) group 2/52 CH + MTA(PBS) and (c) group 12/52 CH + MTA(PBS).

into the experimental groups according to the thickness of the thinnest, most prone to fracture wall, ensured that root dimensions would have as minimal effect on differences amongst the groups as practically achievable. Other studies standardized the external dimensions of the root at the widest portion of the root (the CEJ level), the length of the root and the size of the root canal (Milani *et al.* 2012, EL-Ma'aita *et al.* 2013, Evren *et al.* 2016, Çiçek *et al.* 2017, Aksel *et al.* 2017, Ürkmez & Erdem 2020). During storage, all samples were closed coronally to simulate clinical conditions where phosphate ions are only available through apical diffusion.

Kawamoto *et al.* (2008) suggested that the alkalinity of calcium hydroxide might lead to the breakdown of the inorganic dentine structure or the denaturing of the collagen network, resulting in poorer fracture resistance. Time is required for such an effect to occur because the collagen fibrils are not readily accessible to calcium hydroxide. Penetration of non-setting calcium hydroxide into the human dentine tubules was not detected after 1 month of dressing but could be seen after 3 and 6 months (Twati *et al.* 2009).

There have only been two clinical studies investigating the fracture resistance of traumatized immature teeth that received long-term treatment with calcium hydroxide (Cvek 1972, Al-Jundi 2004). They reported a 40% and 32% incidence of root fracture, respectively. However, confounding factors including cracks and fractures that could have been a result of previous trauma, the stage of root development, the presence of root resorption, a technique for apexification and the type of restoration provided had not been considered.

Fracture resistance of teeth treated with calcium hydroxide has been investigated using a load to failure test and whole roots or teeth in studies using mostly ovine teeth (Andreasen *et al.* 2002, 2006, Hatibović-Kofman *et al.* 2008, Hawkins *et al.* 2015, Kahler *et al.* 2018). Results of those studies should be considered with caution because morphology, chemical composition and physical properties of animal teeth may influence the results (Yassen *et al.* 2011).

Only two studies have used human teeth (Rosenberg *et al.* 2007, Zarei *et al.* 2013). In the study by Rosenberg *et al.* (2007), whole mature teeth were mounted for the fracture test in a different way, with a chisel applied to the cervical surface of the crown perpendicular to the long axis of the tooth. The PDL was not simulated. Furthermore, teeth filled with a sealer and guttapercha rather than empty teeth were used as a control. The results of the study by Zarei *et al.* (2013) revealed that the mean maximum force at fracture was significantly higher in the control group than teeth filled with calcium hydroxide at 1, 3 and 6 months. No difference was found at week one and after 12 months.

Based on the results of the present study, it appears that non-setting calcium hydroxide dressing can be used for up to 12 weeks for root canal disinfection without a negative effect on fracture resistance of human roots. The results are in contrast with the study Zarei *et al.* (2013), but interestingly, in their study the mean compressive force required to fracture those roots seemed to increase with time in both groups, and doubled at 3 months, compared to week one.

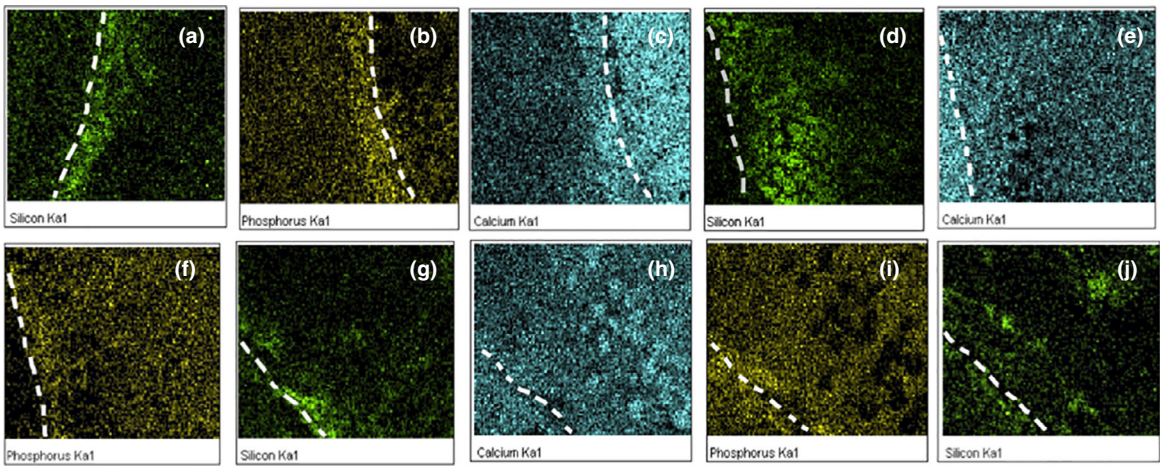

Figure 6 Mapping results for: (a)–(c) group MTA(W), (d)–(f) group 2/52 CH + MTA(PBS), (g)–(i) group 12/52 CH + MTA (PBS) and (j) group MTA(PBS). Dotted line shows the approximate margin of the MTA.

Clinically, phosphate ions are present in dentinal tubules of a tooth, but irrigating solutions used during root canal treatment will reach up to several hundreds of μm into the dentinal tubules (Zou *et al.* 2010, Qian & Haapasalo 2011) and remove them. The intact cementum will prevent phosphate ion diffusion into the dentinal tubules from the soft tissue fluid in the PDL. Therefore, the only source of phosphate ions for roots filled with an MTA cement mixed with water is via direct contact with the periapical tissues or, perhaps very limited, through diffusion from the PDL via a large lateral canal. Since the source of the phosphate ions in dentinal tubules directly adjacent to the MTA is very limited, a limited biomineralization can be expected. This was confirmed in a study by Reyes-Carmona *et al.* (2010b), where the inter-tubular mineralization in dentine adjacent to MTA was only observed in the part of the root adjacent to the source of phosphate ions (Reyes-Carmona *et al.* 2010b). To overcome this and facilitate biomineralization, Ca- and Mg-free PBS was used to mix with MTA cement, in the present study.

This study demonstrated that fracture resistance of human roots treated with MTA mixed with water was significantly higher than those that received irrigation only (negative control). These results confirmed the findings of previous studies on human teeth (Milani *et al.* 2012, EL-Ma'aita *et al.* 2013, Bayram & Bayram 2016, Aksel *et al.* 2017, Ürkmez & Erdem 2020). At least two types of MTA have been tested: white MTA (Angelus Solucoes Odontologicas, Brazil) (Milani *et al.* 2012, Bayram & Bayram 2016) and ProRoot MTA (Dentsply) (EL-Ma'aita *et al.* 2013, Ürkmez & Erdem 2020). Aksel *et al.* (2017) did not state the type of MTA used in their study.

The current study also showed that MTA mixed with PBS has a similar strengthening effect on weak roots, but roots filled with MTA mixed with water may be more prone to fracture at low values of applied stress, which would make them less reliable. No study has investigated fracture resistance of teeth filled with MTA mixed with PBS, against which the findings of the present study can be compared.

The present study found that MTA mixed with water did not improve the fracture resistance of simulated immature roots if they had been previously dressed with non-setting calcium hydroxide for either 2 or 12 weeks. This is in contrast to the findings by Karapinar-Kazandag *et al.* (2016), also carried out on human teeth using the same type of MTA cement but used 1-week CH pre-medication. In their study, the samples were mounted horizontally and loaded perpendicular to their long axis. It could be hypothesized that Ca^{2+} and OH^- ions from calcium hydroxide could diffuse through the dentinal tubules and precipitate as solid calcium hydroxide, and/or when Ca^{2+} ions come into contact with carbon dioxide ions in the air or carbonate ions in tissue fluid, calcium carbonate is formed which has no biological properties (Estrela *et al.* 1999). Calcium carbonate and solid calcium hydroxide deposits could present a chemical and mechanical obstacle to MTA adaptation to the root canal wall (Stefopoulos *et al.* 2008). Furthermore, when MTA mixed with water is used after the

calcium hydroxide dressing, there may be some unhydrated MTA present (Saghiri *et al.* 2009).

In the present study, a significant strengthening effect compared to the roots which received no treatment, and no difference in fracture resistance between roots with apical plugs of MTA mixed with PBS, with and without, a 2-week CH pre-medication was found. This could be explained by the fact that the 2-week contact of calcium hydroxide with dentine was not long enough for it to penetrate human dentine tubules and denature the collagen matrix in dentine to any great extent, and in the presence of phosphate-containing fluid, increased pH and Ca^{2+} concentration may enhance the supersaturation of phosphate-containing fluid and promote calcium phosphate precipitation which could mature into carbonated apatite.

This study showed there was no strengthening effect of MTA mixed with PBS on thin-walled roots when calcium hydroxide pre-medication was used for twelve weeks, although there was no significant difference between groups MTA(PBS) and 12/52CH + MTA (PBS). It has been previously demonstrated that apatite formation by the MTA-PBS system deposited amongst collagen fibrils and triggered the formation of an interfacial layer with tag-like structures at the MTA–dentine interface. The presence of an intact collagen network may, therefore, play an important role in the formation of the tag-like structures (Reyes-Carmona *et al.* 2009). It is likely that the samples in this group had a precipitate in the dentinal tubules after 12 weeks of calcium hydroxide treatment (Twati *et al.* 2009), and the collagen network has been damaged. This is likely to reduce the beneficial effect of the apatite formation on the root dentine.

The SEM images and the results of the element mapping undertaken in the present study seem to support the results of the fracture resistance test and may explain the strengthening effect of some treatment modalities, previously demonstrated by Ürkmez & Erdem (2020). Element diffusion (Si, P and Ca) from the MTA into dentine was only found in samples restored with MTA mixed with PBS, even if calcium hydroxide pre-medication was used, and in samples with MTA mixed with water but only when CH was not used in advance (Fig. 6).

Han & Okiji (2011) investigated the uptake of Ca and Si from white MTA by bovine dentine in the presence of Ca- and Mg-free PBS. Their study found Ca- and Si-rich dentine areas along the MTA–dentine interface. Other studies (Sarkar *et al.* 2005, Reyes-Carmona *et al.* 2010a, Dreger *et al.* 2012) investigated the dentine–MTA interface of human teeth stored in Ca- and Mg-free PBS, or implanted subcutaneously in rats (Dreger *et al.* 2012). After examination using SEM-EDX, the element composition of the interfacial dentine revealed reduced amounts of Si and the presence of P in the IF layer (Sarkar *et al.* 2005). The IF layer contained mainly Ca and P (Reyes-Carmona *et al.* 2010a), and dentine, IF layer and inter-tubular mineralization showed similar composition, although dentine had a reduced amount of Si (Dreger *et al.* 2012).

The dentine–MTA interface of samples exposed to phosphate-containing fluid has been also investigated using optical or scanning electron microscopy. It was possible to clearly distinguish the interfacial layer with tag-like structures entering dentinal tubules (Sarkar *et al.* 2005, Reyes-Carmona *et al.* 2009, 2010a,b, Dreger *et al.* 2012).

The interfacial layer formation observed in samples in Group 2 may be responsible for the superior sealing ability, and prevention of marginal leakage (Martin *et al.* 2007) and material displacement (Reyes-Carmona *et al.* 2010a, de Ameida *et al.* 2014) and improved fracture resistance (Ürkmez & Erdem 2020). This also suggests that a primary monoblock could be achieved in a root canal, which has been challenging in the past (Tay & Pashley 2007), provided enough phosphate ions was available to react with MTA. Because MTA and dentine have similar moduli of elasticity, 15–30 GPa (Tay & Pashley 2007) and 18.6 GPa (Eskitaşcioğlu *et al.* 2002), respectively, a mechanically homogenous unit could be formed in the root. The ability of MTA mixed with Ca- and Mg-free PBS to form a monoblock with human dentine may explain the higher dependability of those samples in comparison to those filled with MTA mixed with water detected by the comparison of the Weibull moduli (Table 4 and Fig. 4).

The present study found that root filling with MTA may have a weakening effect on fracture resistance of roots that were previously treated with calcium hydroxide for 12 weeks (Tables 4 and 6). This could be caused by the more severe denaturing effect on the collagen fibrils by further exposure to OH^- produced during the hydration of MTA (Yaltirik *et al.* 2004, Sawyer *et al.* 2012) and released for extended periods (Fridland & Rosado 2003, Heward & Sedgley 2011, Leiendecker *et al.* 2012).

The results of the present study suggest that the type of treatment a root receives may influence the type of fracture. A split was the most common type of fracture in the study (69% overall), similar to the findings of Aksel *et al.* (2017), and was particularly common in groups 2/52CH and 12/52CH + MTA(W) (94% in both), and in the negative control (78%). The results of this study agree the results of EL-Ma'aita *et al.* (2013), from this it is evident that some treatment modalities can support the root and bond it until the force is high enough to fracture the tooth into several pieces.

The results showed also that type of treatment may have an influence on the depth of root fracture, which was in contrast to the findings by Karapinar-Kazandag *et al.* (2016), who examined fracture resistance of roots filled with MTA after 1-week CH pre-medication, intact roots and immature unfilled roots. In the present study, 72.5% of all samples suffered deep fractures across the root (either below the cylinder level or VRF), which clinically would be below the alveolar bone level. The present study found no significant difference between the mean maximum force for fracture types and depths. It appears that factors other than the applied force may predispose endodontically treated teeth to a certain type and depth of fracture (Kishen 2015). The results suggest that calcium hydroxide pre-medication affects the properties of MTA when the cement is mixed with water. In clinical practice, subject to satisfactory clinical evaluation, if calcium hydroxide pre-medication is required, it should be used short term (2 weeks), and MTA mixed with Ca- and Mg-free PBS could be used as an apical barrier to strengthen weak roots. If, however, calcium hydroxide dressing is required for longer than 2 weeks, other ways of root strengthening should be considered (Lui 1994, Cauwels *et al.* 2014, Brito-Júnior *et al.* 2014, Dikbas *et al.* 2014).

More research is needed on the chemical and physical properties on MTA mixed with PBS, and on the effect of calcium hydroxide pre-medication on MTA-type cements, to establish a clinical protocol for the management of structurally weak roots disinfected with calcium hydroxide, and reduce the risk of catastrophic root fractures.

Conclusions

Mineral trioxide aggregate mixed with Ca- and Mg-free phosphate-buffered saline had a significant strengthening effect on the fracture resistance of structurally weak roots, even when short-term calcium hydroxide pre-medication had been used. MTA mixed with water lost its strengthening effect on human roots when 2- or 12-week calcium hydroxide pre-treatment had been used. Use of calcium hydroxide intra-canal dressing for up to 12 weeks had no negative effect on fracture resistance of human roots.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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